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Prevalence of seed-borne fungi of different vegetables seeds in Bangladesh

Ratan Chandro Mohanto¹, Saleh Ahmed Shahriar^{2*},
 Abu Noman Faruq Ahmmed¹, Farzana Afrin Nishi³,
 Most. Nurjahan Khatun Eaty⁴

¹Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, ²Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, ³Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, ⁴Faculty of Agriculture, International University of Business Agriculture and Technology, Dhaka, Bangladesh

ABSTRACT

The experiment was carried out to evaluate the prevalence of seed-borne fungi of ten selected vegetables seeds e.g. amaranth, Indian spinach, bottle gourd, sweet gourd, snake gourd, okra, bitter gourd, cucumber, brinjal and country bean. Untreated and unknown grower's bulk vegetables loose seeds were collected from three different seed sources of Rangpur district, Bangladesh e.g. New Bangla Seeds; Islam Seeds and Rafiq Traders. The high level of infection was found in the seeds of Rafiq Traders and the lowest infection was observed in the seeds of New Bangla Seeds. Islam Seeds provided moderate infection of fungal pathogens. Fungal fruiting bodies, mycelium (white and cottony) and acervuli were found under microscopic observation. The observed physical abnormalities viz. deformed, shrinkage, swelling, spotted and undersized seeds were recorded from all seed sources. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp., *Alternaria* sp., *Chaetomium* sp., *Rhizopus* sp. and unidentified bacteria were observed by blotter method. *Aspergillus flavus* showed maximum incidence (9.0%) in okra and minimum incidence (5.50%) in brinjal. *Aspergillus niger* was the most predominant (9.0%) in sweet gourd and minor (5.0%) in bottle gourd. Incidence of *Fusarium* sp. (5.0%) and *Rhizopus* sp. (8.75%) were predominant in bottle gourd. The lowest incidence for *Fusarium* sp. (2.50%) and *Rhizopus* sp. (3.0%) were found in snake gourd and amaranth respectively. The highest incidence of *Alternaria* sp. (4.75%) and *Chaetomium* sp. (9.0%) were found both in sweet gourd and bitter gourd but minimum incidence of *Alternaria* sp. (2.0%) and *Chaetomium* (3.75%) were recorded in amaranth and okra respectively. In case of unidentified bacteria, maximum incidence (4.50%) was recorded in bottle gourd.

KEYWORDS: Vegetables seeds, seed-borne fungi, incidence, prevalence, blotter method

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*Corresponding author:
 Saleh Ahmed Shahriar
 E-mail: shahriar777.ss@gmail.com
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INTRODUCTION

Seed is the biological objected input in agriculture. Quality of seed has the pre-requisite of seed as a planting material for sowing in land in order to get disease free seedling and plant and finally to achieve satisfactory yield [1,2]. The major constraints to vegetables production in Bangladesh are; insects, weeds, diseases, environmental factors, marketing, postharvest losses etc. Amongst these production constraints, pest and diseases are the major one. Among them, infection by fungi, bacteria, nematodes or viruses are major [3,4]. Therefore, diseases act as the main limiting factors to economic production of vegetables. Over 200 diseases have been reported to affect the different vegetables in the world. Among them the seed-borne pathogens mostly fungi play a vital role in disease development [5,6]. Farmers have to deal with significant losses due to infections by serious seed-borne fungi on their plants, which may start from germinating seed, seedling in the nursery, matured plants

in the field and proceed till the products are harvested, fruits and seeds stored. Significant crop losses due to seed-borne pathogens have been recorded [7,8]. Seed health and quality is an important factor in the control of diseases, since an infected seed is less viable, has low germination, reduced vigor and reduced yield [9].

From our perspective, the overall health status and quality of loose seeds of vegetables were not up to the mark. This is because, saprophytic pathogenic genera were observed with the seeds that may deteriorate the health status and quality of seeds and seedlings. Therefore, the disease incidence percentage and infection of seed-borne fungi have to be evaluated based on the seeds of vegetables and the seed sources. The experiment aimed at evaluating the disease incidence level of seed-borne fungi of different vegetables seeds and their prevalence against seed borne-fungi.

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MATERIALS AND METHODS

Sampling

The experiment was conducted in Seed Health Laboratory of the Department of Plant Pathology of Shere-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh. The experiment was conducted during the period from November, 2016 to April, 2017. Excluding tested, treated, procured, packed and imported seeds only growers bulk seeds of ten vegetables were collected and preserved by the seed trader for the sac to the farmers were collected from three seed stores namely New Bangla seed, Islam Seed and Rafiq Traders of Rangpur district in Bangladesh for conducting blotter method to examine prevalence of seed-borne fungi of different vegetables seed. The samples were brought directly to the Laboratory and kept them in air tight until use for subsequent studies. Two leafy vegetables (amaranth and Indian spinach), seven fruit vegetables (bottle gourd, sweet gourd, cucumber, bitter gourd, snake gourd, okra and brinjal) and one pod vegetable (country bean) were evaluated to determine the pathogenic fungal infection in loose vegetables seeds. Three primary samples of each vegetable were collected from each source. The composite sample was formed by combination and mixing all the primary samples taken from the seed lot or containers or bag [8].

Detection and Identification of Fungi

Seed samples were used for blotter test for detection of seed-borne fungi of vegetables seeds. In this incubation method, 9 cm diameter pyrex glass petridish and whatman no. 1 filter paper were used. 400 seeds were taken randomly from each sample and were placed in 10 petridishes (10 seeds per petridish). The petridishes with seeds were then incubated at 22°C temperature under 12 hours for 7 days in the Laboratory [1,2].

Blotter Method

In seed health testing Blotter method was used [8,10].

Analysis of Data

The laboratory experiment was conducted by following Complete Randomized Design (CRD) with three replications. The data obtained for different characters were statistically analyzed by “MSTAT-C” program to find out the significance of the different levels of loose seeds collected from three seed sources.

RESULTS AND DISCUSSIONS

Prevalence of Seed-borne Fungi Identified by Blotter Method

Identified characteristics for all seed-borne fungi are presented in Figure 1.

Seed-borne Fungal Infections

The highest (7.50%) disease incidence in amaranth was observed by *Aspergillus flavus* and *Aspergillus niger* and the lowest (2.0%) disease incidence was observed by *Alternaria sp.* The highest (8.0%) disease incidence in Indian spinach was observed by *Aspergillus flavus* and *Aspergillus niger* and the lowest (2.25%) disease incidence was observed by *Alternaria sp.* The highest (8.0%) disease incidence in bottle gourd was observed by *Aspergillus flavus* and the lowest (2.75%) disease incidence was observed by *Fusarium sp.* and *Alternaria sp.* The highest (9.0%) disease incidence in sweet gourd was observed by *Aspergillus niger* and *Chaetomium sp.* and the lowest (4.25%) disease incidence was observed by *Fusarium sp.* and *Alternaria sp.* The highest (9.0%) disease incidence in bitter gourd was observed by *Chaetomium sp.* and the lowest (3.0%) disease incidence was observed by *Fusarium sp.* The highest (6.75%) disease incidence in cucumber was observed by *Rhizopus sp.* and the lowest (3.0%) disease incidence was observed by *Alternaria sp.* The highest (8.50%) disease incidence in snake gourd was observed by *Aspergillus flavus* and *Chaetomium sp.* and the lowest (2.50%) disease incidence was observed by *Fusarium sp.* The highest (9.0%) disease incidence in okra was observed by *Aspergillus flavus* and the lowest (3.50%) disease incidence was observed by *Alternaria sp.* The highest (6.75%) disease incidence in brinjal was observed by *Aspergillus flavus* and the lowest (3.50%) disease incidence was observed by *Alternaria sp.* The highest (8.50%) disease incidence in country bean was observed by *Aspergillus niger* and the lowest (3.75%) disease incidence was observed by *Alternaria sp.* The data are presented in Table 1.

Fungi Identified and their Frequency of Occurrence

Aspergillus flavus, *Aspergillus niger*, *Fusarium sp.*, *Alternaria sp.*, *Chaetomium sp.*, *Rhizopus sp.* and unidentified bacteria were observed by blotter method. In case of amaranth, incidence of different seed-borne fungi e.g. *Aspergillus flavus* (6.25-7.50%), *Aspergillus niger* (6.25-7.50%), *Fusarium sp.* (2.75-3.50%), *Alternaria sp.* (2.0-4.0%), *Chaetomium sp.* (5.0-6.50%) and *Rhizopus sp.* (3.0-4.0%) were observed and unidentified bacteria was 1.75-2.25%. For Indian spinach, pathogen incidence level of *Aspergillus flavus* (6.50-8.0%), *Aspergillus niger* (5.75-8.0%), *Fusarium sp.* (2.87-3.50%), *Alternaria sp.* (2.25-2.50%), *Chaetomium sp.* (5.50-6.0%), *Rhizopus sp.* (5.25-6.0%) were identified and unidentified bacteria was 2.25-3.50%. For bottle gourd, incidence of identified seed-borne fungi were *Aspergillus flavus* (6.0-8.0%), *Aspergillus niger* was (5.0-6.25%), *Fusarium sp.* (2.75-4.0%), *Alternaria sp.* (2.75-4.25%), *Chaetomium sp.* (4.50-5.75%), *Rhizopus sp.* (5.0-6.25%) and unidentified bacteria was 1.50-4.50%. In case of sweet gourd, different Incidence level for seed-borne fungi *Aspergillus flavus* (7.75-8.50%), *Aspergillus niger* (8.25-9.0%), *Fusarium sp.* (4.25-5.0%), *Alternaria sp.* (4.25-4.75%), *Chaetomium sp.* (8.0-9.0%), *Rhizopus sp.* (7.50-8.75%) were detected and unidentified bacteria was 3.75-4.25%. For bitter gourd, incidence of different seed-borne fungi e.g. *Aspergillus flavus* (7.25-8.0%), *Aspergillus niger* (5.75-7.0%), *Fusarium sp.* (3.0-3.75%), *Alternaria sp.* (3.75-4.75%), *Chaetomium sp.* (6.75-9.0%), *Rhizopus sp.*

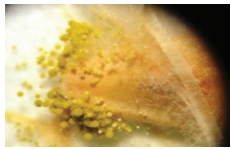

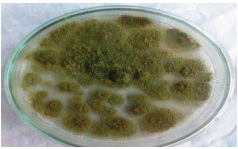

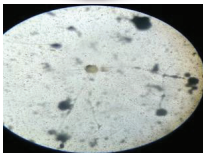
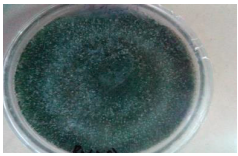

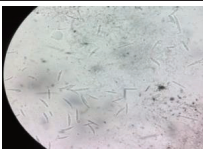
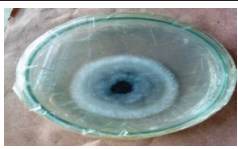
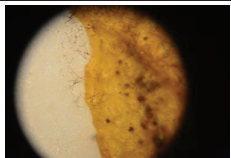

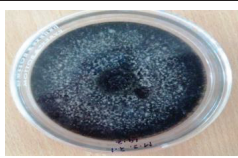
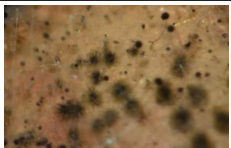
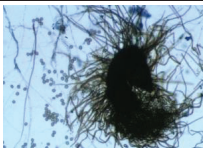
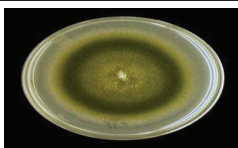
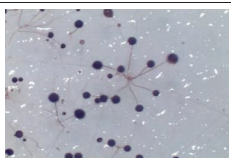


Fungi	Stereomicroscopic View	Compound microscopic view(40x)	Pure culture on PDA medium
<i>Aspergillus flavus</i>			
<i>Aspergillus niger</i>			
<i>Fusarium</i> sp.			
<i>Alternaria</i> sp.			
<i>Chaetomium</i> Sp.			
<i>Rizopus</i> sp.			

Figure 1: Seed-borne fungi identified by blotter method

(4.0-6.50%) were found and unidentified bacteria was 3.50-4.25%. In case of cucumber, incidence of indetified seed-borne fungi were *Aspergillus flavus* (5.53-6.50%), *Aspergillus niger* (6.0-6.50%), *Fusarium* sp. (3.25-4.0%), *Alternaria* sp. (3.0-3.75%), *Chaetomium* sp. (5.75-6.25%), *Rhizopus* sp. (4.75-6.75%) and unidentified bacteria was 3.0-4.25%. Due to infection of different seed-borne fungi, in snake gourd incidence of *Aspergillus flavus* (7.25-8.50%), *Aspergillus niger* (6.0-6.50%), *Fusarium* sp. (2.50-4.0%), *Alternaria* sp. (3.0-3.50%), *Chaetomium* sp. (6.50-8.50%), *Rhizopus* sp. (5.75-6.75%) were observed and unidentified bacteria was 2.25-2.75%. For okra, incidence of identified seed-borne fungi were *Aspergillus flavus* (7.50-9.0%), *Aspergillus niger* (7.5-8.75%), *Fusarium* sp. (3.75-4.0%), *Alternaria* sp. (3.5-4.5%), *Chaetomium* sp. (3.75-6.25%), *Rhizopus* sp. (4.0-5.25%) and unidentified bacteria was 2.75-3.75%. In case of brinjal, incidence of seed-borne fungi e.g. *Aspergillus flavus* (5.50-6.75%), *Aspergillus niger* (5.25-6.50%), *Fusarium* sp. (4.0-4.25%), *Alternaria* sp. (3.5-4.0%), *Chaetomium* sp. (4.50-6.25%), *Rhizopus* sp. (3.75-5.0%) were found and unidentified bacteria was 2.75-3.25%. For country bean, incidence of identified seed-borne fungi e.g. *Aspergillus*

flavus (7.0-8.0%), *Aspergillus niger* (6.0-8.5%), *Fusarium* sp. (4.25-4.75%), *Alternaria* sp. (3.75-4.75%), *Chaetomium* sp. (7.25-8.25%), *Rhizopus* sp. (6.25-7.50%) were detected and unidentified bacteria was 2.50-3.87%. The data are presented in Table 1.

Health Status of Seeds

Germination of vegetable seeds recorded by blotter method were varied significantly. Germination was highest in amaranth (94%) and lowest in Indian spinach (72%). There was a highly positive relationship between germination failure and prevalence of seed-borne fungal infections. Normal seedlings ranged from 56-84% in 10 vegetables. Normal seedlings were found highest in amaranth (84%), whereas abnormal seedlings ranged from 5-21.5%. Non-germinated seeds like hard and rotten were found to be ranged from 2-18% and 4-15%, respectively in seeds of 10 vegetables. The notorious fungi, *Fusarium* sp. cause seedlings infection or foot and root rot disease. *Fusarium* sp. is a well-known seed-borne pathogen able of causing germination failure/seed rot, damping-off, root rot and wilts etc. in many crops [2,10]. The cause of germination failure was pathogenic

Table 1. Prevalence of seed-borne fungi identified by blotter method

Source name	% Pathogen incidence							% seed infection
	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Fusarium</i> sp.	<i>Alternaria</i> sp.	<i>Chaetomium</i> spp.	<i>Rhizopus</i> sp.	Unidentified bacteria	
<i>Amaranth (Amaranthus spinosus)</i>								
New Bangla seeds	7.50a	7.50a	3.50a	3.50a	6.50a	4.00a	1.75b	34.25
Islam seeds	6.25b	6.25b	2.75b	4.00a	5.00b	3.00b	2.25a	29.5
Rafiq traders	7.25a	7.25a	3.50a	2.00b	6.50a	3.00b	2.25a	31.75
LSD	0.997	0.843	0.596	0.565	0.843	0.653	0.460	
Level of significance	*	**	*	**	**	**	*	
CV %	8.91	7.53	11.47	11.16	8.78	12.25	13.86	
<i>Indian spinach (Basella alba)</i>								
New Bangla seeds	8.00a	5.75b	3.50a	2.50a	6.00ab	6.00a	2.25b	34
Islam seeds	6.50b	8.00a	2.87a	2.87a	6.75a	5.25a	3.50a	35.62
Rafiq traders	8.00a	7.25a	3.25a	3.25a	5.50b	5.25a	2.75b	34.22
LSD	1.25	1.06	0.741	0.741	0.884	1.13	0.532	
Level of significance	*	**	Ns	Ns	*	Ns	**	
CV %	10.42	9.52	14.46	13.33	9.09	12.86	11.76	
<i>Bottle gourd (Lagenaria siceraria)</i>								
New Bangla seeds	6.5b	5.75ab	4.00a	4.25a	5.25ab	5.50ab	4.50a	35.75
Islam seeds	6.0b	6.25a	2.75b	3.50b	5.75a	6.25a	2.50b	33
Rafiq traders	8.0a	5.0b	3.25b	2.75c	4.50b	5.00b	1.50c	30
LSD	1.193	0.997	0.532	0.653	0.753	0.799	0.653	
Level of significance	**	**	**	**	**	**	**	
CV %	10.91	11.0	10.0	11.66	9.12	8.96	10.50	
<i>Sweet gourd (Cucurbita moschata)</i>								
New Bangla seeds	8.25a	9.00a	4.50a	4.25a	9.00a	8.75a	3.75a	47.5
Islam seeds	8.50a	8.25b	4.25a	4.50a	8.25a	7.50a	4.00a	45.25
Rafiq traders	7.75a	9.00a	5.00a	4.75a	8.00a	7.75a	4.25a	46.5
LSD	1.25	0.704	0.884	0.923	1.16	1.25	0.843	
Level of significance	Ns	**	Ns	Ns	Ns	**	**	
CV %	9.57	5.04	10.06	8.83	8.63	9.77	11.18	
<i>Bitter gourd (Momordica charantia)</i>								
New Bangla seeds	7.25a	6.75a	3.25ab	3.75b	7.50b	4.00c	3.50b	36
Islam seeds	7.50a	5.75b	3.00 b	3.75b	6.75b	5.25b	3.50b	35.5
Rafiq traders	8.00a	7.00a	3.75 a	4.75a	9.00a	6.50a	4.25a	43.25
LSD	0.884	0.843	0.532	0.596	1.16	0.799	0.704	
Level of significance	Ns	**	**	**	**	**	**	
CV %	7.29	8.11	10.00	9.13	9.37	9.52	11.76	
<i>Cucumber (Cucumis sativus)</i>								
New Bangla seeds	6.50a	6.50a	4.00a	3.25ab	5.75a	4.75b	3.00b	33.75
Islam seeds	5.53b	6.00a	3.25b	3.00b	6.00a	6.25a	3.75ab	33.78
Rafiq traders	5.75b	6.25a	3.75ab	3.75a	6.25a	6.75a	4.25a	36.75
LSD	0.706	0.596	0.653	0.653	0.843	0.884	0.753	
Level of significance	*	Ns	*	*	Ns	**	**	
CV %	7.46	5.96	11.13	12.25	8.78	9.34	10.86	
<i>Snake gourd (Trichosanthes cucumerina)</i>								
New Bangla seeds	7.25a	6.00a	2.50b	3.00a	7.25b	5.75b	2.75a	34.5
Islam seeds	8.50a	6.50a	4.00a	3.00a	6.50b	6.25ab	2.25b	37
Rafiq traders	8.50a	6.00a	3.75a	3.50a	8.50a	6.75a	2.25b	39.25
LSD	1.27	0.753	0.704	0.653	0.884	0.799	0.460	
Level of significance	Ns	Ns	**	*	**	*	*	
CV %	9.89	7.64	12.91	12.89	7.45	8.00	11.95	
<i>Okra (Abelmoschus esculentus)</i>								
New Bangla seeds	9.00a	8.75a	4.00 a	4.50a	5.50a	4.75ab	3.75a	40.25
Islam seeds	8.00ab	8.00ab	4.00a	3.50b	6.25a	5.25a	3.50a	38.5
Rafiq traders	7.50b	7.50b	3.75a	3.50b	3.75b	4.00b	2.75b	32.75
LSD	1.35	1.03	0.596	0.843	0.843	0.843	0.532	
Level of significance	*	*	Ns	*	**	*	**	
CV %	10.41	7.99	9.52	13.75	10.20	11.29	10.00	
<i>Brinjal (Solanum melongena)</i>								
New Bangla seeds	5.75b	6.50a	4.25a	3.50a	5.50a	4.75a	2.75b	33
Islam seeds	6.75a	5.25b	4.01a	3.50a	4.50b	3.75b	2.75b	30.6
Rafiq traders	5.50b	6.00ab	4.00a	4.00a	6.25a	5.00a	3.25a	34
LSD	0.843	0.799	0.715	0.653	0.961	0.753	0.460	
Level of significance	**	**	Ns	Ns	**	**	*	
CV %	8.78	8.45	10.95	11.13	11.09	10.48	9.90	

(Contd...)

Table 1. (Continued)

Source name	% Pathogen incidence							% seed infection
	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Fusarium</i> sp.	<i>Alternaria</i> sp.	<i>Chaetomium</i> spp.	<i>Rhizopus</i> sp.	Unidentified bacteria	
	Country bean (<i>Phaseolus vulgaris</i>)							
New Bangla seeds	8.00a	6.00b	4.75a	3.75b	8.25a	6.25b	3.87a	40.87
Islam seeds	7.00a	8.50a	4.75a	4.00b	7.25a	7.50a	2.50b	41.5
Rafiq traders	7.75a	8.50a	4.25a	4.75a	7.75a	7.00ab	3.75a	43.75
LSD	1.38	0.753	0.799	0.653	0.961	1.09	0.639	
Level of significance	Ns	**	Ns	**	Ns	**	**	
CV %	11.42	6.15	10.91	9.80	7.75	9.94	11.84	

Ns=Non-significant, * = Significant at 5% level of significance as per DMRT, ** = Significant at 1% level of significance as per DMRT

infection and sometimes it was physiological cause. Among the 10 vegetables seeds the vigor index was ranged from 677.3 to 2747. The above study exposed that very good connection between seed-borne fungal infections and germination failure of vegetable seeds existed [1].

Two leafy vegetables (amaranth and Indian spinach), seven fruit vegetables (bottle gourd, sweet gourd, cucumber, bitter gourd, snake gourd, okra and brinjal) and one pod vegetable (country bean) were evaluated to determine prevalence of seed-borne fungi of loose vegetables seeds. A considerable amount of seed-borne pathogenic fungi and unidentified bacteria were observed by blotter method. In blotter method, five seed-borne pathogenic genera namely *Aspergillus*, *Fusarium*, *Alternaria*, *Chaetomium*, *Rhizopus* and unidentified bacterium were identified in 10 vegetables seeds. The present investigation revealed that altogether six different fungi viz. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp., *Alternaria* sp., *Chaetomium* sp., and *Rhizopus* sp. were found to be associated with Amaranth seeds. There are previous reports of seed-borne fungi in different seeds like bottle gourd [11]. Similar result was reported by some earlier workers [2,7]. Yesuf *et al.* [12] reported that seed-borne fungi on bean (*Phaseolus vulgaris*) and most common fungi were isolated viz. *Alternaria* sp. (75 %), *Aspergillus* (73%), *Rhizopus* sp. (73%), *Cladosporium* sp. (98%), *Penicillium* sp. (69%), *Fusarium* sp. (38 %).

High quality seed is not only important for increasing crop production but also for proper establishment of sound seed industry in the country. Seed is a common carrier of plant pathogens and pathogen free seed is the important input material for agriculture [13]. The present experiment showed that a lot of seed-borne fungi were associated with loose vegetables seeds. *Aspergillus flavus* showed almost the highest disease incidence level and *Alternaria* sp. showed the lowest disease incidence level for all these collected vegetables seeds. Sweet gourd showed maximum and amaranth showed minimum vulnerability for all these seed-borne fungi. Seed-borne fungi may be appeared due to improper management of vegetables seeds in storage condition. Considering the overall findings it was exposed that the seed health status and quality of loose vegetables seeds were not a satisfactory level. Farmers are therefore advised to collect the seeds from reliable source, check their health status and quality and seed treatment should be done before sowing in the main field.

AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration with all authors. Authors RCM, SAS, FAN and MNKE designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed review of literature. Author ANFA supervised the experiment. Author SAS supervised and edited the manuscript. All authors read and approved the final manuscript.

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